Expression and modulation of Wnt, Bmp, and putative antagonist genes in the bovine caruncle endometrium during the implantation stage

Aires, M.B.; Degaki, K.Y.; Dantzer, Vibeke; Spencer, T.E.; Yamada, A.T.

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[P08.08].
CATHEPSIN B, CATHEPSIN L AND CYSTATIN C IN PORCINE UTERI AND PLACENTAE: A MODEL FOR PROTEIN MODIFICATION DURING UTILIZATION AND FLUID-PHASE TRANSPORT ACROSS UTERINE EPITHELIA, AREOLAE AND NEONATAL GUT

G.A. Johnson*[1], G. Song[2], D.W. Bailey[1], K.A. Dunlap[1], R.C. Burghardt[1], F.W. Bazer[2]. [1]Department of Veterinary Integrative Biosciences, Texas A&M University, United States, [2]Department of Animal Science, Texas A&M University, United States

Proteases, Cathepsins (CTSB) and (CTSL1), and their inhibitors, Cystatin C (CST3), remodel endometrium and placenta for transplacental transport of gases, micronutrients and macromolecules. We examined *CTSB*, *CTSL1* and *CST3* expression and hormonal regulation in endometria and placentae of pigs. 1) gilts were hysterectomized on Day 9, 12, or 15 of the estrous cycle, or Day 9, 12, 15, 20, 25, 30, 35, 40, 50, 60, or 85 of pregnancy. 2) cyclic gilts were injected (Days 11-14) with estrogen (E2) and hysterectomized on Day 15 or 90 of pseudopregnancy. 3) gilts were ovariectomized on Day 12, injected with progesterone (P4) for 28 days, and hysterectomized on Day 40. *CTSB* increased in luminal epithelium (LE) after Day 30 of gestation. *CTSB* in LE was not affected by E2, but was increased by P4. *CTSB* was abundant in chorionic epithelium by Day 20 and remained through Day 85. *CTSL1* increased in LE, GE and trophectoderm by Day 20 of gestation. Endometrial *CTSL1* was not affected by E2, but was increased by P4. *CTSL1* was highly expressed in the chorionic epithelia that form areolae to absorb secretions of uterine glands. *CST3* was expressed in presumptive immune cells within endometria, but increased in LE by Day 25 of gestation. *CST3* was not affected by E2 at Day 15, but increased in LE on Day 90 of pseudopregnancy. P4 decreased *CST3* in LE, but increased expression in immune cells. We next examined *CTSL1* in the jejunum of neonatal pigs which absorbs IgG for passive immunity. *CTSL1* was expressed in enterocytes of villi. These results show that CTSL1 is expressed by areolae, GE, and neonatal intestine, each of which have roles in fluid-phase transport of proteins, and that interactions between CTSL1, CST3 and CTSB may modify proteins during utilization and transport across uterine, placental and gut epithelia.

Keywords: Pig, proteases, areolae, uterus

[P08.09].
CAVEOLAE AND CAVEOLINS IN THE CLONED TRANSGENIC CATTLE PLACENTA

FTV Pereira*[1], FV Meirelles[2], F Bressan[2], M Miranda[2], AC Assis Neto[1], MA Miglino[2]. [1]Paulista State University, Brazil, [2]University of Sao Paulo, Brazil

Caveolae and caveolin-mediated endocytosis are internalization pathways in the placental transport. However, their roles in a cattle placenta have not been addressed. In this study, we compared the involvement of caveolae- and caveolins -1,-2 in cloned transgenic cattle. Fetal fibroblasts expressing the GFP gene were used as nuclei donors to cloning by nuclear transfer (NT), to produce the gestations by embryo transfer. Transmission electron microscopy (TEM) and immunhistochemistry (IHC – anti-caveolins -1, -2) were performed on placentomes and chorioallantois from 5 cloned (60 and 90 days of gestation) and 10 controls in the same gestation period. The tissues were glutaraldehyde or formalin fixed. At the TEM we could observe and characterize the structures called caveolae in blood capillaries of the chorioallantoic membrane and placentomes by natural (control) and cloned transgenic cattle gestation. The caveolae appears as a plasmaleme vesicles and invaginations in both of the plasmic membrane in luminal and abluminal portion. However, we have observed many microvilli in the trophoblast of the placentome, such as in the chorioallantoic membrane. The fetal and maternal villi were immunoreactive to caveolin-1, but the strongest reaction was observed in the endometrial stroma. The chorioallantois trophoblast and placentome were immunoreactive to caveolin -2, mainly in a trophoblast giant binucleate cell. The results obtained by the TEM and IHC indicated that the caveolae plus microvilli are very important sites on placental transport and signaling in the natural and cloned transgenic cattle gestation.

Funded: FAPESP.
Keywords: Caveolae, Caveolins, Cloned cattle, Transgenic

[P08.10].
EXPRESSION AND MODULATION OF Wnt, Bmp, AND PUTATIVE ANTAGONIST GENES IN THE BOVINE CARUNCLE ENDOMETRIUM DURING THE IMPLANTATION STAGE

M B Aires[1], K Y Degaki[1], V Dantzer[2], T E Spencer[3], A T Yamada*[1]. [1]University of Campinas, Brazil, [2]Faculty of Life Sciences, Denmark, [3]Texas A&M University, United States

Development of villous-like projection on cow caruncle (CAR) surface as local endometrium response to establish the embryo-chorion anchorage site for synepithelialchorial placentation, closely resembles epithelial-mesenchyme interaction during embryo organogenesis of gut, urinay and respiratory mucosa that are strictly controlled by morphogens like WNT and BMP proteins families. The present work comparatively evaluated the evolution of villous-like projection in the pregnant and non-pregnat (NP) cow (*Bos* spp) caruncle based on morphological analysis and, *Wnt* and *Bmp* morphogen gene families expression by PCR and *in situ* hybridization. By histological analysis of CAR mucosa during implantation was established four developmental stages: S1–trophoblast cells uterine epithelium adhesion, S2– initial villous-like projections on the CAR surface, S3– expansion and anastomoses of villous-like projections, and S4– placentome consolidation. Anti-PCNA immunostaining demonstrated intense proliferative activity from S1toS4 on CAR epithelial and stromal cells, resulting in a 5-fold expansion of endometrial tissue. By RT-PCR and *in situ* hybridization, the expression of the *Bmp2* and *Wnt7a* genes was seen up-regulated in the CAR, with the highest expression level detected at S1 compared to NP. Otherwise, *Wnt2* and *Wnt5a* expression levels were lower in the CAR. *Dkk1* and *Sfrp2* were widely expressed in the LP and S1-S4 CAR and IC 45 endometrium. *Noggin* expression was consistently lower in the CAR, whereas *Sostdc1* expression was higher in the CAR than IC at S1 and then declined in both regions until S4. Therefore, simultaneous expression of *Wnt* and *Bmp* together with their antagonists in the CAR and IC regions seems to be responsible for the capability of cow endometrium quick remodeling and ability to develop synepithelialchorial placentation. Furthermore, they also attest to the complex synergistic/antagonistic signaling mechanisms of several still unknown factors modulating the cow uterine mucosa response during embryo implantation and synepithelialchorial placentation. Grants: CNPq and CAPES.

Keywords: placentome, caruncle endometrium, morphogen, WNT and BMP